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14. ABSTRACT

Our research efforts to date suggest that both OC use and childbearing are protective in *BRCA1/2* carriers and non-carriers. Our findings also suggest that there are unknown genetic and/or environmental factors that alter the risk of ovarian cancer, especially among BRCA1/2 carriers. It is possible that genetic variations in the inflammation pathway may be one such genetic factor and that exposure to inflammation-associated factors may be one such environmental factor. This research has produced 3 abstracts, 1 journal article, 1 manuscript under review and resulted in further funding from the NCI to investigate the role of modifiers of ovarian cancer risk in BRCA1/2 carriers.

15. SUBJECT TERMS
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INTRODUCTION:

The goal of this project is to determine whether oral contraceptives (OCs) and parity are as protective against ovarian cancer in BRCA1/2 carriers as they are for women in general (Specific Aim 1). The second goal is to determine whether there existed genetic polymorphisms that may account for differences in survival between BRCA1/2 carriers and non-carriers (Specific Aim 2). For Specific Aim 1, we identified Jewish women with epithelial ovarian cancer. We genotyped these women for the 3 BRCA1/2 mutations found in Ashkenazi women. We compared oral contraceptive use and parity between carriers and non-carriers. For Specific Aim 2, we obtained DNA on Jewish Israeli women with ovarian cancer who were genotyped for the 3 Jewish mutations and for whom survival data was available. We attempted to identify polymorphisms in the inflammation pathway that could explain the greater survival in BRCA1/2 carriers.

BODY:

This research had two specific aims. Specific aim 1 was accomplished and resulted in three conference presentations (1,3), one publication (2), and one manuscript under review (4) (see appendix 1).

Specific aim 2 has not been accomplished. We received 637 DNA specimens from our collaborators in Israel. Although most of the initial 96 test specimens appeared to be of high quality, most of the remaining specimens failed to genotype. We tried several methods to amplify and clean the DNA but were unsuccessful. At this time, our Israeli colleagues have committed to reisolating DNA from their stored specimens, ensuring its quality and reshipping the specimens to us. Our laboratory collaborator has also agreed to test the samples and, if they work, he will genotype them for polymorphisms in the inflammation pathway.

In the interim, we have used our resources to identify differences in ovarian cancer risk between Israeli Jewish women and American women in order to focus our explorations. Our results to date (4) show that factors protective in general (oral contraceptive use, bearing children, tubal ligation, hysterectomy, no talc use) are protective in both the US and Israeli population. However, the magnitude of the protection appears greater for American women than for Israeli women. Our findings together with the greater percentage of BRCA1/2 carriage among women in Israel would suggest that the rate of ovarian cancer would be greater in Israel than in the US. To the contrary, the rate of ovarian cancer is remarkably similar in the two countries. We conclude that it is likely that there is some genetic and/or environmental factor that may provide protection to Israeli Jewish women. We are hopeful that the DNA we receive from Israel will work and will allow us to examine the possibility of genetic modifying factors. Further evidence for our conclusion comes from data in which we show that among Jewish women with ovarian cancer, the percent of carriers of a BRCA1/2 mutation is greater for US women than for Israeli Jewish women (46% vs. 25%). This suggests an environmental factor.

Much attention has focused on the role of inflammation in ovarian cancer risk and survival. Thus, we pilot tested our laboratory assays for a set of polymorphisms in inflammation-associated genes (IL1A-4845 T/G, IL1A-M889 T/C, IL1B-3957 G/A, IL6-M174 C/G, IL10-M819 T/C, IL10-M1082 C/T, IL18-M137 G/C) in a convenience sample of 141 ovarian cancer cases. Our assays were successful and our preliminary data suggested that the IL18-M137 SNP may be associated with tumor stage and histologic subtype, suggesting that this polymorphism may influence disease phenotype and survival. It further suggests that exposure to inflammation-associated factors may alter the risk of ovarian cancer in women with a certain genetic makeup.

KEY RESEARCH ACCOMPLISHMENTS:

- showed that oral contraceptive use and bearing children are as protective in BRCA1/2 carriers as they are in women in general
- showed that the incidence rates of ovarian cancer are similar in Israel and in Western Pennsylvania (USA), despite the greater prevalence of BRCA1/2 mutations in Israel. This suggests that Israeli women more often engage in protective behaviors (or US women more often engage in risk-associated behaviors) or that protective factors are more protective in the Israeli population.
- showed that oral contraceptives, bearing children, having a tubal ligation and having a hysterectomy protect against ovarian cancer in a population at a genetically high risk (Jewish Israeli women) as well as in the general American population, while talc use and greater BMI is a risk factor for both populations.
- showed that OC use and bearing children are *more* protective in the US population, suggesting that unknown genetic and/or environmental factors exist for ovarian cancer
- showed that Jewish women with ovarian in the US are more likely to be BRCA1/2 carriers than Jewish women with ovarian cancer in Israel, suggesting that BRCA1/2 is more penetrant in the US. This supports the existence of an unknown environmental factor.
- showed that genetic variants in inflammation-associated genes may affect ovarian cancer phenotype, suggesting that these variants may prove promising for investigating the genetic factors that might affect BRCA1/2 penetrance. These data further suggest that inflammation-associated environmental exposures (e.g., NSAID use) may be fruitful areas of investigation for factors that alter ovarian cancer risk in BRCA1/2 carriers.

REPORTABLE OUTCOMES:

Abstracts

Roxana Moslehi, Francesmary **Modugno,** Roberta B. Ness, Steven Narod.: Reproductive Factors and Ovarian Cancer Risk in Jewish BRCA1 and BRCA2 Mutation Carriers. In *Proceedings of the American Society of Human Genetics Annual Meeting.* San Diego, CA, October 2001. Also in *American Journal of Human Genetics* 69(4):274 Abstract 534.

S. Sadetzki, F. **Modugno**, B Oberman, A. Chetrit F. Lubin, R.B. Ness. Risk factors for ovarian cancer- is there a missing link? In *Proceedings of the American Society of Clinical Oncology Annual Meeting 2004.* New Orleans, LA. June 2004. Also in the *Proceedings of the European Congress of Epidemiology.* Porto, Portugal. September 2004.

Journal Articles

Francesmary **Modugno,** Roxanna Moslehi, Roberta B Ness, Deborah Brookes Nelson, Steven Belle, Jeffrey Kant, James Wheeler, Aimee Wonderlick, David Fishman, Beth Karlan, Harvey Risch, Daniel Cramer, Marie-Pierre Dube, Steven Narod. Reproductive Factors and Ovarian Cancer Risk in Jewish BRCA1 and BRCA2 Mutation Carriers. *Cancer Causes and Control* 2003; 14(5):439-446.

Manuscripts under Review

Siegal Sadetzki, Francesmary **Modugno**, Bernice Oberman, Flora Lubin, Angela Chetrit, Robert B. Ness. Risk Factors for Ovarian Cancer- Is There a Missing Link? (*note: Dr. Sadetzki and I contributed equally to this paper).*

Grants

Based on this work, Dr. Modugno applied for and received funding from the NCI to pursue additional endpoints examining risk modifiers of ovarian cancer in BRCA1/2 carriers (R03CA92776).

Talks

Exploring the Roles of Hormones and Inflammation in Ovarian Cancer Epidemiology. Women's Cancer Research Seminar Series. Ovarian Cancer Center of Excellence, Magee-Womens Hospital

Ovarian Cancer Risk Factors: Putting the Pieces Together. Senior Vice Chancellor Research Seminar, University of Pittsburgh

CONCLUSIONS:

In conclusion, our initial findings suggest that both OC use and childbearing are protective in *BRCA1/2* carriers and non-carriers. Our findings also suggest that there are unknown genetic and/or environmental factors that alter the risk of ovarian cancer. It is possible that genetic variations in the inflammation pathway may be one such genetic factor and that exposure to inflammation-associated factors may be one such environmental factor.

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1. Roxana Moslehi, Francesmary **Modugno,** Roberta B. Ness, Steven Narod.: Reproductive Factors and Ovarian Cancer Risk in Jewish BRCA1 and BRCA2 Mutation Carriers. In *Proceedings of the American Society of Human Genetics Annual Meeting.* San Diego, CA, October 2001. Also in *American Journal of Human Genetics* 69(4):274 Abstract 534.

2. Francesmary **Modugno,** Roxanna Moslehi, Roberta B Ness, Deborah Brookes Nelson, Steven Belle, Jeffrey Kant, James Wheeler, Aimee Wonderlick, David Fishman, Beth Karlan, Harvey Risch, Daniel Cramer, Marie-Pierre Dube, Steven Narod. Reproductive Factors and Ovarian Cancer Risk in Jewish BRCA1 and BRCA2 Mutation Carriers. *Cancer Causes and Control* 2003; 14(5):439-446.

3. S. Sadetzki, F. **Modugno**, B Oberman, A. Chetrit F. Lubin, R.B. Ness. Risk factors for ovarian cancer- is there a missing link? In *Proceedings of the American Society of Clinical Oncology Annual Meeting 2004.* New Orleans, LA. June 2004. Also in the *Proceedings of the European Congress of Epidemiology.* Porto, Portugal. September 2004.

4. Siegal Sadetzki, Francesmary **Modugno**, Bernice Oberman, Flora Lubin, Angela Chetrit, Robert B. Ness. Risk Factors for Ovarian Cancer- Is There a Missing Link? (*note: Dr. Sadetzki and I contributed equally to this paper).*

APPENDICES:

Published article: Modugno et al. Cancer Causes and Control 2003.
Manuscript Sadetzki, Modugno et al. Under Review

Personnel Supported

Jeff Kant, MD - laboratory assays
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Cancer Causes and Control **14**: 439–446, 2003.

Reproductive factors and ovarian cancer risk in Jewish *BRCA1* and *BRCA2* mutation carriers (United States)

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Key words: *BRCA1*, oral contraceptives, ovarian cancer, parity.

Abstract

Objective: To determine whether oral contraceptive (OC) use, childbearing, breastfeeding and tubal ligation differ between ovarian cancer cases with and without a *BRCA1/2* mutation.

Methods: A case-only study of 242 Jewish women with invasive epithelial ovarian cancer. Women were genotyped for three Ashkenazi founder mutations (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*). We obtained data on OC use, childbearing, breastfeeding, gynecologic surgeries and other reproductive factors from each woman. We compared the frequencies of these risk factors in carriers and non-carriers using unconditional logistic-regression, controlling for other covariates.

Results: Among the 242 cases, 64 (26.4%) carried one of the *BRCA1* founder mutations, and 31 (12.8%) carried the *BRCA2* mutation. Although there were no differences in the percent of nulliparous women between carriers and non-carriers, parous *BRCA1* carriers reported fewer live births than non-carriers (average of 2.1 *versus* 2.5 live births, OR = 0.61, 95%CI = 0.39–0.95, adjusted for age at diagnosis, tubal ligation and duration of OC use). Carriers and non-carriers did not differ in their history of breastfeeding, or in their lifetime use of OCs. *BRCA1* carriers were more likely than non-carriers to have had a tubal ligation (25.0 *versus* 10.2%, OR = 3.67, 95%CI = 1.55–8.70, adjusted for age at diagnosis, number of live births and OC duration).

Conclusions: In general, OC use, childbearing and breastfeeding do not differ between *BRCA1/2* carriers and non-carriers with ovarian cancer. However, the effects of tubal ligation may differ between *BRCA1* carriers and non-carriers.

Introduction

Mortality from invasive ovarian cancer is very high, with a five year survival rate of approximately 40% [1]. Survival is better with early stage disease, but the majority of patients present with metastatic disease [1]. To date, no effective early detection techniques have been identified and primary prevention represents an important opportunity for reducing ovarian cancer morbidity and mortality. Women with mutations in the cancer predisposing *BRCA1* and *BRCA2* genes have a lifetime ovarian cancer risk of 16–36% [2–5]. Using oral contraceptives (OCs), bearing children and

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breast-feeding have consistently been shown to reduce ovarian cancer risk among women in general [6, 7]. Tubal ligation has also been shown to reduce ovarian cancer risk [6, 8]. However, little is known about the impact of these factors on ovarian cancer risk in *BRCA1/2* mutation carriers. In a case–control study comparing 207 women with hereditary ovarian cancer to 161 of their unaffected sisters without the disease, OC use was less common among women with the disease [9]. This suggests that OC use may reduce the risk of ovarian cancer in women with a mutation in the *BRCA1* or *BRCA2* genes. However, the results of that study have been questioned, because the *BRCA1/2* carrier status of some of the sisters was unknown. This unknown data can potentially invalidate the findings. More recently, a case–control study of Israeli Jewish women found that the risk of ovarian cancer among carriers of a *BRCA1* or *BRCA2* mutation decreases with each birth but not with increased duration of use of oral contraceptives [10]. These conflicting data suggest the need to further investigate the potential of OCs as a chemopreventive agent among women with a *BRCA1/2* mutation.

In this study, we aimed to evaluate the potential benefit associated with OC use among women at high risk for ovarian cancer because they carry a mutated *BRCA1* or *BRCA2* gene. We also sought to determine the benefit or risk associated with other reproductive factors, including childbearing, breastfeeding, and tubal ligation in these women.

Methods

Subjects

Because of the high prevalence of three *BRCA1/2* founder mutations among Ashkenazi Jewish women with invasive epithelial ovarian cancer [11], we limited our study to Jewish women with epithelial ovarian cancer and with no prior history of breast cancer. Data on subjects were pooled from four sources: two population-based case–control studies of epithelial ovarian cancer in the United States (100 cases) [12, 13], a hospital-based study of Jewish women with epithelial ovarian cancer among 11 centers in North America and Israel (208 cases) [11], and a genetic counseling center in Chicago (14 cases). The Chicago clinic had been one of the sites for the hospital-based study, but the 14 incident, invasive cases included in this analysis were in addition to those participating in the original study. There was some overlap between cases included in the current study and those in the previous report of OCs and ovarian cancer in *BRCA1/2* carriers [9] but this was less than 10%. Unfortunately, because subject links from the original studies to this study were not maintained, we were unable to identify which cases included in this study were also included in the previous report of OCs and ovarian cancer [9].

Moslehi *et al.* [11] (the hospital-based study) classified a woman as Jewish if three out of four grandparents were Jewish. Questions about place of birth of parents and grandparents further identified Ashkenazi women in that study. In Lu *et al.* [13] (one of the population-based studies), a woman was considered to be Jewish if she indicated that her childhood religious upbringing was Jewish. For the other two sources of subjects, a woman was considered to be Jewish if she classified herself as Jewish on medical records.

Specific descriptions of each study methodology are provided in the original publications [11–13] and are summarized in Table 1. Briefly, Moslehi *et al.* [11] used medical records to identify 465 Jewish women with ovarian cancer. Of these, 80 women were dead, 33 women were found not to have invasive disease on pathology review, 98 women were unreachable, and 49 women refused to participate. The remaining 208 (44.7%) women completed an in-person interview and provided a blood sample. Ness *et al.* [12] identified all women age 20–69 diagnosed with ovarian cancer in the Delaware Valley between 1994 and 1998. Of the 957 eligible women, 69 were too ill to participate, 15 were untraceable, and 92 refused to participate. Fourteen physicians did not consent to their patients' participating, for a total of 767 (80.1%) eligible women who completed an in-person interview. For the study presented here, we used medical records to identify successfully the religious affiliation of 437 of the 767 women, 46 of whom were Jewish, and we used banked pathology specimens (normal tissue blocks) to determine *BRCA1/2* carrier status of 36 of these women. Lu *et al.* [13] used tumor registries to identify 1080 women with ovarian cancer in eastern Massachusetts and New Hampshire between May 1992 and March 1997. Of the 1080 women, 203 had died or were unreachable, 126 were not contacted because their physician denied permission, 136 women declined participation, and 52 had non-epithelial ovarian cancer. The remaining 563 (52%) women were interviewed, during which time they provided a blood sample and answered questions about their childhood religious upbringing. Of the 563 women, 54 identified Jewish as the religion of their upbringing.

Each study obtained written informed consent from participants and was approved by the appropriate institutional review boards.

Table 1. Summary recruitment and eligibility characteristics of four pooled studies

Study	Moslehi *et al.* [11]	Ness *et al.* [12]	Lue *et al.* [13]	Chicago	Present study
Year of diagnosis	1980–1999[a]	1993–1998	1992–1997	1990–1999	1990–1999
Place	11 centers in North America and Israel	Delaware Valley, USA	Massachusetts and New Hampshire	Chicago	na
Total eligible	465	957	1080	14	na
Total participants	208	767 (437 with known religion)	563	14	na
Method of determining Jewish descent	3 of 4 grandparents Jewish	Self disclosure of current religion	Childhood upbringing	Self disclosure of current religion	na
Total eligible Jewish women	208	46	54	14	322
Total with complete exposure data	191	46	54	14	305
Total with *BRCA1/2* status known	191	36[b]	54	14	295
Total confirmed invasive	162	31	35	14	242
Total with *BRCA1/2* founder mutation	65	15	13	2	95

[a] 26/162 final participants included in this analysis were from 1980–1989; 1 final participant was from 1972.
[b] Ten tissue blocks were unobtained.

Exposure information, BRCA1/2 mutation status and data quality

From each study source, data were requested on the use of OCs, including age at first and last use, and duration of use. Data were also obtained on number of live births, age at first and last live birth, and total duration of breastfeeding. We further requested information on other factors including age at menarche, body mass index, history of hysterectomy and history of tubal ligation. Because data on age at menopause and hormone replacement therapy were inconsistent among the studies, we were not able to include them in our analyses. We obtained details of tumor histology on all subjects, and we restricted our analyses to invasive ovarian cancers of the epithelial type. All data were checked for internal consistency and corrections or clarifications were requested from the original investigators when necessary.

All subjects were screened for the three Ashkenazi founder mutations (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*) Mutation analysis was performed by the original study investigators using several established detection techniques, including heteroduplex analysis, single-strand conformation analysis and allele-specific oligonucleotide hybridization. In addition, Moslehi *et al.* [11] tested all subjects for mutations in exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2* using the protein-truncation test [14]. Truncating mutations in these exons represent about 70% of the *BRCA1/2* mutations found to date [11]. No women from that study included in the analysis reported here were found to have any *BRCA1/2* mutations other than one of the three *BRCA1/2* founder mutations. Regardless of the technique employed, all mutations were confirmed by direct sequencing of DNA. Non-carriers were defined as women with none of the three mutations (for the studies employing only the Ashkenazi panel) and no other detected mutations (for subjects from Moslehi *et al.* [11]). *BRCA1* carriers were defined as women with either the 185delAG or the 5382insC in *BRCA1*. Women with the 6174delT in *BRCA2* were defined as *BRCA2* mutation carriers.

All subject data submitted for the pooled analysis were anonymous. Approval for the pooled analysis was obtained from the University of Pittsburgh Institutional Review Board.

Study design and statistical analyses

To determine whether carriers and non-carriers differed in OC use, parity, breast-feeding, and tubal ligation, we employed a case-only study design [15]. In a case-only study, cases with the genotype (carriers) form the 'pseudo-cases' and cases without the susceptibility genotype (non-carriers) form the 'pseudo-control' group. The two groups are compared with respect to the prevalence of each exposure. The odds ratio (OR) reflects the association between the exposure and the genotype (assuming independence of genotype and exposure). If this ratio is different from one, then the relative risk associated with the exposure differs for carriers and non-carriers. For a protective factor such as OC use, childbearing and breastfeeding in ovarian cancer, an OR greater than one indicates that the factor was more prevalent among the carriers ('pseudo-cases');

thus, the factor provides less protection to carriers than to non-carriers. Conversely, an OR of less than one indicates that the factor was less prevalent among the carriers, and suggests that the factor provides greater protection for carriers than for non-carriers.

To control for potentially confounding effects of other factors, we used unconditional logistic-regression analyses and included as covariates age at diagnosis and year of birth as continuous terms. Age at diagnosis was included in all models because univariate analyses showed a significant difference between carriers and non-carriers. Because the studies differed in the time period in which they were performed, year of birth was included in order to control for secular trends in OC use, parity and breastfeeding. However, there were no differences in results between analyses including year of birth and those excluding the variable. We therefore present the most parsimonious model in this paper. To check the reasonableness of pooling data from diverse sources, we calculated a Mantel–Haenszel test for heterogeneity for all major results. In none of the associations between BRCA status and reproductive factors did we find statistically significant heterogeneity among subject source. In addition, models that included a variable for study site did not differ in results from models excluding the variable; thus, the final models presented in this paper do not include a variable for study site. All analyses were performed with the STATA statistical software package (STATA Corporation, Release 5.0) and all p values given are from two-sided tests.

We analyzed all cases with complete exposure data. Because one of the parent studies [11] noted a difference in age at onset between *BRCA1* and *BRCA2* carriers, and because univariate analyses showed other differences in risk factors between *BRCA1* and *BRCA2* carriers for the entire study population, we analyzed the data for *BRCA1* and *BRCA2* carriers both jointly and separately.

Results

Table 1 presents a summary of the subjects participating in this study from the four parent studies. A total of 322 cases of epithelial ovarian cancer in Jewish women were identified. Of these, complete exposure data were obtained on 305 cases and *BRCA1/2* status was confirmed on 295 cases. Of the 295 cases, invasive histology was confirmed on all but 53 cases, for a total of 242 cases included in this analysis.

The characteristics of the 64 subjects with a *BRCA1* mutation, the 31 subjects with a *BRCA2* mutation, and the 147 non-carriers are presented in Table 2. As expected, *BRCA1* carriers with invasive tumors were diagnosed at a significantly earlier average age than non-carriers (51.2 *versus* 57.5 years, $p = 0.001$). In contrast, *BRCA2* carriers were diagnosed at later ages than non-carriers (60.8 *versus* 57.5 years), although this difference was not significant. The difference in age at diagnosis between *BRCA1* carriers and *BRCA2* carriers, however, was significant ($p < 0.001$).

Only 11.7% of non-carriers reported a family history of ovarian cancer, compared to 16.1% of *BRCA1* carriers ($p = 0.39$) and 29.0% of *BRCA2* carriers ($p = 0.017$ for comparison to non-carriers). Similarly, non-carriers were less likely to report a family history of breast cancer (15.2% for non-carriers *versus* 22.6% for *BRCA1* carriers and 35.5% for *BRCA2* carriers). The difference between *BRCA2* carriers and the non-carriers was significant ($p = 0.011$).

Table 3 compares reproductive factors among carriers and non-carriers. After adjusting for possible confounders, there were no significant differences between the groups for age at menarche, ages at first and last live birth, or breastfeeding. There was also no difference in the percent of nulliparous women between carriers and non-carriers. However, parous *BRCA1* carriers reported fewer live births than parous non-carriers. The average number of live births among parous women was 2.5 among non-carriers, but only 2.1 among *BRCA1* carriers (OR = 0.61, 95%CI = 0.39–0.95, adjusted for age at diagnosis, tubal ligation and duration of OC use). Although parous *BRCA2* carriers also reported fewer live births than non-carriers, the difference between parous *BRCA2* carriers and non-carriers was not significant.

Interestingly, compared to non-carriers, *BRCA1* carriers were more likely to report having had a tubal ligation (25.0 *versus* 10.2%, OR = 3.67, 95%CI = 1.55–8.70 adjusted for age at diagnosis, number of live births and OC use). *BRCA2* carriers were less likely to report a history of tubal ligation compared to non-carriers, but the difference was not significant. However, the difference between *BRCA1* and *BRCA2* carriers was significant ($p < 0.05$). No differences in hysterectomy were found between carriers and non-carriers.

We compared additional characteristics of oral contraceptive use between carriers and non-carriers (Table 4). No significant differences were found in ever use of OCs or in duration of OC use. However, *BRCA1* carriers were likely to have begun using OCs at a later mean age than non-carriers (24.0 *versus* 23.2 years of age, OR = 1.15, 95%CI = 1.01–1.30 adjusted for age at diagnosis, number of live births, tubal ligation and OC duration). *BRCA1* carriers were also more likely to report recent use of OCs. The mean interval from last

Table 2. Characteristics of *BRCA1* and *BRCA2* carriers and non-carriers

	BRCA− (n = 147)	*BRCA1*+ (n = 64)		*BRCA2*+ (n = 31)	
Demographic characteristics					
Mean year of birth	1936 ± 12.8	**1941 ± 9.9**	**$p = 0.005$**	1932 ± 12.1	$p = 0.14$
Mean age at diagnosis (years)	57.5 ± 12.5	**51.2 ± 9.9**	**$p = 0.001$**	60.8 ± 11.3	$p = 0.20$
Mean body mass index (kg/m^2)	24.8 ± 5.6	25.1 ± 5.5	$p = 0.753$	25.8 ± 6.8	$p = 0.40$
Family history of ovarian cancer, n (%)	17 (11.7)	10 (16.1)	$p = 0.391$	**9 (29.0)**	**$p = 0.017$**
Family history of breast cancer, n (%)	22 (15.2)	14 (22.6)	$p = 0.200$	**11 (35.5)**	**$p = 0.011$**
Family history of ovarian or breast cancer, n (%)	26 (17.7)	16 (25.0)	$p = 0.223$	**11 (35.5)**	**$p = 0.030$**
Reproductive characteristics					
Mean age at menarche (years)	12.7 ± 1.4	12.6 ± 1.6	$p = 0.746$	12.3 ± 1.6	$p = 0.139$
Parous, n (%)	124 (84.4)	49 (76.6)	$p = 0.178$	29 (93.6)	$p = 0.196$
Mean number of livebirths[a]	2.5 ± 1.2	**2.1 ± 0.8**	$p = 0.027$	2.3 ± 0.9	$p = 0.508$
Mean age at first birth[a]	26.0 ± 4.7	26.6 ± 4.2	$p = 0.453$	27.1 ± 5.1	$p = 0.261$
Mean age at last birth[a]	31.3 ± 4.8	30.1 ± 4.3	$p = 0.159$	32.1 ± 5.4	$p = 0.388$
Mean time since first birth (years)[a]	32.7 ± 13.8	**26.9 ± 11.6**	$p = 0.012$	33.6 ± 11.8	$p = 0.747$
Mean time since last birth (years)[a]	27.4 ± 12.9	23.3 ± 11.7	$p = 0.061$	28.6 ± 11.0	$p = 0.646$
Breastfeeding, n (%)	47 (32.0)	22 (34.4)	$p = 0.732$	8 (25.8)	$p = 0.501$
Mean duration of breastfeeding (months)[a]	5.6 ± 16.2	6.5 ± 16.4	$p = 0.743$	6.8 ± 12.3	$p = 0.721$
Tubal Ligation, n (%)	15 (10.2)	**16 (25.0)**	**$p = 0.007$**	2 (6.5)	$p = 0.522$
Hysterectomy, n (%)	19 (12.9)	7 (10.9)	$p = 0.687$	6 (19.4)	$p = 0.353$
OC characteristics					
OC use, n (%)	58 (39.2)	**36 (56.3)**	**$p = 0.025$**	11 (35.5)	$p = 0.680$
Mean duration of use (years)[b]	5.1 ± 4.9	3.7 ± 3.6	$p = 0.163$	3.4 ± 5.2	$p = 0.294$
Mean age at first use[b]	23.2 ± 4.9	24.0 ± 5.3	$p = 0.425$	23.6 ± 6.2	$p = 0.801$
Mean time since last use (years)[b]	21.4 ± 9.1	19.6 ± 8.0	$p = 0.360$	23.8 ± 9.9	$p = 0.429$

Plus–minus values are means ± SD. *p*-values are for comparison of carriers to non-carriers.

Bolded entries are significant at $p < 0.05$.

Missing data are as follows: four subjects (2 BRCA−, 2 *BRCA1*+): family history of breast or ovarian cancers; 1 *BRCA2*+ subject: BMI; 1 BRCA−subject: age at first and last birth.

[a] Among women who had a live birth; [b] Among ever users.

Table 3. Adjusted ORs and 95% CIs for reproductive characteristics according to *BRCA1/2* carrier status

	BRCA− (n = 147)	*BRCA1/2*+ (all carriers combined) (n = 95)		*BRCA1*+ (n = 64)		*BRCA2*+ (n = 31)	
		Adj[a] OR	95% CI	Adj[a] OR	95% CI	Adj[a] OR	95% CI
Age at menarche	Referent	0.93	0.78–1.11	1.01	0.81–1.26	0.80	0.60–1.06
Parous[b]	Referent	0.89	0.42–1.87	0.67	0.29–1.52	2.50	0.54–11.68
Number of livebirths[b,d]	Referent	**0.70**	**0.49–0.99**	**0.61**	**0.39–0.95**	0.85	0.55–1.31
Age at first birth[d]	Referent	1.02	0.95–1.09	0.98	0.90–1.07	1.05	0.96–1.15
Age at last birth[d]	Referent	1.01	0.94–1.08	0.96	0.88–1.04	1.06	0.97–1.16
Time since first birth[d]	Referent	0.98	0.92–1.06	1.02	0.94–1.11	0.95	0.87–1.04
Time since last birth[d]	Referent	1.00	0.93–1.06	1.04	0.96–1.13	0.95	0.86–1.03
Breastfeed	Referent	1.09	0.61–1.97	1.36	0.68–2.73	0.70	0.28–1.72
Duration of breastfeeding[d]	Referent	1.02	0.99–1.04	1.01	0.98–1.04	1.02	0.99–1.05
Tubal ligation[c]	Referent	**2.32**	**1.06–5.11**	**3.67**	**1.55–8.70**	0.65	0.14–3.16
Hysterectomy	Referent	1.56	0.69–3.54	1.79	0.63–5.07	1.37	0.48–3.91

[a] Each row represents a separate model. All models were adjusted for age at diagnosis, number of live births (continuous variables) and OC use and history of tubal ligation (yes/no), except for those noted by ([b]), which were not adjusted for number of live births, and those noted by ([c]), which were not adjusted for tubal ligation. ORs in bold are significant at the $p < 0.05$ level.

[d] Among women who had a live birth.

use to diagnosis was 19.6 years for *BRCA1* carriers and 21.4 years for non-carriers (OR = 0.89, 95%CI = 0.79–0.99 adjusted for age at diagnosis, number of live births, tubal ligation and OC duration). The differences in age at first OC use or recent OC use between *BRCA2* carriers and non-carriers were not significant.

Table 4. Adjusted ORs and 95% CIs for OC use according to *BRCA1* and *BRCA2* carrier status

	BRCA– (n = 147)	*BRCA1/2*+ (all carriers combined) (n = 95)		*BRCA1*+ (n = 64)		*BRCA2*+ (n = 31)	
		Adj[a] OR	95% CI	Adj[a] OR	95% CI	Adj[a] OR	95% CI
OC use	Referent	1.21	0.67–2.17	1.29	0.66–2.52	1.11	0.44–2.76
Duration of use (years)[b,c]	Referent	0.93	0.83–1.03	0.92	0.80–1.04	0.92	0.78–1.09
Age at first use[c]	Referent	1.11	0.99–1.24	**1.15**	**1.01–1.30**	0.96	0.80–1.16
Time since last use[c]	Referent	0.91	0.82–1.01	**0.89**	**0.79–0.99**	1.01	0.86–1.19

[a] Each row represents a separate model. Each model is adjusted for age at diagnosis, year of birth, number of live births, OC duration (continuous variables) and history of tubal ligation, except for that noted by ([b]), which was adjusted for OC duration. ORs in bold are significant at the $p < 0.05$ level.

[c] Among ever users.

Discussion

We pooled data on Jewish women with invasive ovarian cancer from four sources in order to determine whether there were differences in OC use, childbearing, breastfeeding and tubal ligation between *BRCA1/2* mutation carriers with invasive ovarian cancer and non-carriers with the disease.

We found no difference in the percent of nulliparous women between carriers and non-carriers, although parous *BRCA1* carriers had experienced fewer live births than non-carriers (2.1 *versus* 2.5). This suggests that the effect of bearing children is similar for both *BRCA1* carriers and non-carriers. It is possible that the earlier age at diagnosis among *BRCA1* carriers may partially explain fewer live births in that group. However, in our population-based case–control study [16], healthy controls with a mean age of 49.5 years had on average 2.8 live births. This suggests that the earlier age of diagnosis cannot fully explain the observed reduced parity. Moreover, our analyses showed a similar finding (fewer live births compared to non-carriers) for parous *BRCA2* carriers, despite that fact that compared with non-carriers and *BRCA1* carriers, *BRCA2* carriers had a later age at diagnosis. We are careful to note, however, that this result failed to reach statistical significance, possibly due to the small number of *BRCA2* carriers in our study.

With regards to breastfeeding, we found no differences between *BRCA1/2* carriers and non-carriers. Thus, the effect of breastfeeding on ovarian cancer risk appears to be similar for both carriers and non-carriers. Similarly, no difference between *BRCA1/2* carriers and non-carriers were found for ever having a hysterectomy, suggesting that the effect of hysterectomy on ovarian cancer risk does not differ between carriers and non-carriers.

We found that OC use also appeared to be similar for both carriers and non-carriers, confirming a previous report [9]. Although we did find a statistically significant difference in age at first use and recency of use between *BRCA1* carriers and non-carriers, these differences were small and may be due to chance. We failed to demonstrate a similar association between early OC use or recency of OC use for *BRCA2* carriers. Again, this may be due to differences in the effects of OC use in *BRCA2* carriers, or it may be due to the small number of *BRCA2* carriers in our study.

We further found that the protection associated with early OC use differed between *BRCA1* and *BRCA2*, although this difference may be due to the small number of *BRCA2* carriers. Notably, the direction of the ORs for the age and timing data among *BRCA2* carriers was opposite to that of the ORs for the *BRCA1* carriers, suggesting that the difference between the two groups may be real and not an artifact of sample size.

These results are in contrast to those of Modan *et al.* [10] who reported that the use of OC provided no protection to Israeli Jewish *BRCA1/2* carriers. While we cannot exclude the possibility that our finding is due to chance, we believe that there are differences between the two studies that may explain these disparate findings. In particular, the duration and frequency of use of OCs were far less in the Israeli population than in the population studied here. Moreover, there may be differences in OC formulations between the two populations. In addition, as discussed below, the differences between the study designs (case–control *versus* case-only) and our small sample size may account for the different findings.

Interestingly, *BRCA1* carriers were more likely to report having had a tubal ligation than non-carriers. Several studies have shown an association between tubal ligation and a reduction in ovarian cancer risk [8, 17–19], although the exact mechanism remains unknown. Our results suggest that if the procedure does protect against ovarian cancer, it may not provide the same degree of protection to *BRCA1* carriers. This finding is in contrast to those of Narod *et al.* [20] who report a

reduction in risk from tubal ligation among *BRCA1* carriers (OR = 0.39, 95%CI = 0.21–0.63, adjusted for OC use, parity, history of breast cancer and ethnic group). Data from that study were obtained from a database containing information on women from high-risk families in Canada, the United States and the United Kingdom. The differences between that study and the results presented here may be due to differences in study populations (high-risk women with any *BRCA1/2* mutation *versus* Ashkenazi Jewish women with one of three mutations), study design (matched case–control *versus* case-only), or chance. In particular, the *BRCA1* gene has over 850 known mutations, and it is unknown whether risk factors for ovarian cancer vary by mutation type. Confounding with other factors, such as family history of breast or ovarian cancer, may also explain our findings.

Care must be taken in interpreting our results. First, subjects were drawn from several sources. It is possible that the different study designs and data collection methods could have resulted in differences among the data sets that would affect our results. We note that while tests for heterogeneity between BRCA status and reproductive factors revealed no significant heterogeneity among subject source, it is possible that the tests may be underpowered in this instance because of the small sample size and the amount of stratification needed to perform the analyses. Therefore, such a test may not be very meaningful.

Second, we tested for a subset of mutations associated with ovarian cancer within a well-defined ethnic population. This raises the question of the generalizability of our results to the non-Jewish population or to women with other mutations.

Third, three of the four sources, which provided 80 subjects (33% of the data) for this study, tested for only the three mutations found in the Ashkenazim. Recently, Frank *et al.* [21] reported that among 322 Ashkenazi individuals who underwent full sequence analysis only after negative results from a three-mutation test, six (1.9%) carried a non-founder deleterious mutation. Therefore, we may have missed some mutations and classified some carriers as non-carriers, although in light of the Frank data, we would expect that number to be less than three. Moreover, the study providing the majority of cases [11] tested for most of the truncating mutations in *BRCA1* and *BRCA2* reported to date in addition to the three founder mutations analyzed here. No additional mutations were found. That is, no mutations (other than the three founder mutations) were identified among the subjects reported here. Therefore, the occurrence of carrier misclassification would likely be small. Assuming that this misclassification is non-differential with respect to the exposures we examined, it would bias our results towards the null value.

About 40% of the cases included in this study were interviewed more than one year after their diagnoses. Women with a *BRCA1* or *BRCA2* mutation may have improved survival compared to women with non-hereditary ovarian cancer [22]. Therefore, it is possible that mutation carriers would be over represented among those interviewed more than a year after diagnosis. Indeed, among those women interviewed more than one year after diagnosis, 43% were *BRCA1/2* mutation carriers; among women interviewed within one year of diagnosis, only 36% carried a mutation. However, this methodological issue would only impact our findings if OC use, parity, breastfeeding and/or tubal ligation affect prognosis.

An additional limitation of this study is the sample size, which limits the detectable differences in OC use, parity and other factors between carriers and non-carriers, and may explain some of our negative findings.

Finally, our choice of a case-only approach has limitations that may have affected our findings. In particular, the case-only design assumes independence between the genetic marker and the environmental exposure [15]. However, it is often difficult to make this assessment, even in a large-scale study [10]. Hence, in the absence of such evidence, as is the case here, point estimates and confidence intervals must be interpreted cautiously. In particular, if there is uncertainty about the assumption that OC use and parity are independent of carrier status among Jewish women, then it is possible that the estimates reported here are less precise than the data suggest [23]. The estimates may also be biased. Specifically, if there were a positive association between genotype and exposure in the underlying population, then the interaction OR above one would be biased towards one when compared to the ratio of relative risks that we are attempting to estimate. A case–control analysis would address these limitations. Unfortunately, because our data came from four sources with separate study designs, we lacked a valid control group to which we could compare the distribution of risk factors found among the different case groups. Moreover, because of the low prevalence of *BRCA1/2* mutations in the general population, it is unlikely that we would have had enough carriers in any control population to employ a standard interaction analysis.

In conclusion, our data suggest that in women with ovarian cancer, using oral contraceptives, bearing children and breastfeeding do not differ between women with and without a *BRCA1/2* mutation. While the data presented here confirm previous findings [9], they stand in contrast to those reported recently by Modan *et al.* [10] which suggested that OCs may not be protective in

women with a *BRCA1* or *BRCA2* mutation. Moreover, our results contradict the recent report that tubal ligation provides protection against ovarian cancer in *BRCA1* mutation carriers [20]. The disagreement between our study and these other studies on the protectiveness of OCs and tubal ligation indicate a substantial lack of clarity on how to counsel women at high risk for ovarian cancer.

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Risk Factors for Ovarian Cancer- Is There a Missing Link?

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ABSTRACT

Despite the higher prevalence of *BRCA1/2* mutations among Ashkenazi women, ovarian cancer incidence among Israeli women, European-American born, is similar to that among Caucasians in the US. We explored whether differential exposure to known ovarian cancer risk/protective factors might explain this enigma.

We compared the frequency of, magnitude of association, and attributable risk for known ovarian cancer risk factors between Israeli-Jewish and US Pennsylvanian women using data obtained by personal interviews from two population-based case-control studies conducted in the 1990s. The study groups comprised 998 and 767 cases and 1528 and 1367 controls in the Israeli and American studies respectively.

Significant differences in the distribution of most study variables were found between the groups. The protective roles of oral contraceptive use and parity were more pronounced for Americans (oral contraception for 10+ years vs. never use OR= 0.71 and 0.36, p=0.07; 3+ livebirths vs none OR= 0.49 and 0.26, p=0.03 among Israelis and Americans respectively). Population attributable risks for most factors suggested more protection afforded among American women.

These findings raise the possibility of a modification effect of *BRCA1/2* mutations on ovarian cancer risk factors, or of as-yet unidentified environmental and/or genetic factors that provide increased protection to Israeli women.

Keywords: case-control studies; Israel; ovarian neoplasms; risk factors; United States

INTRODUCTION

Carrying a mutated *BRCA1/2* gene is the strongest known risk factor for the development of ovarian neoplasms (1, 2). The lifetime risk of developing ovarian cancer among non-carriers is 1.6 % compared to 20-50% among carriers (3, 4). While the prevalence of founder mutations in *BRCA1/2* genes among Ashkenazi Jewish women is 2.4%, the prevalence of a mutation in the *BRCA1/2* genes in the general U.S. population is only about 0.16% (3, 5). Thus, one would expect that rates of ovarian cancer in Israel, a country with a majority of Ashkenazi Jews, would be higher than in the U.S. where Ashkenazi Jews are a distinct minority. Surprisingly, the age standardized incidence rates of ovarian cancer among Caucasians in the U.S. (Surveillance, Epidemiology, and End Results (SEER) data) and in Israeli Jewish women (Israeli Cancer Registry) are quite comparable: 13.2 per 100,000 versus 12.7 per 100,000 in 1993-1997, respectively (6).

Three hypotheses may explain the unexpectedly similar ovarian cancer incidence rates between Israeli and American women. First, Israeli women could more often engage in protective behaviors and/or American women could more often be exposed to risk factors. This could lead to similar incidence rates due to different prevalence of known non-genetic exposures in each population. Second, risk factors might affect each population differently. That is, the relative risk for known exposures in Americans might exceed the risk attributable to that risk factor among Israelis. Together, these first two possibilities would be expected to result in higher exposure-related attributable risks among Americans. Albeit not a testable hypothesis, a final possibility is that unidentified exogenous risk factors or genetic factors may differ between Israeli and U.S. populations.

To address the testable hypotheses, we compared the magnitude and effects of known risk and protective factors for ovarian cancer between Israeli-Jewish and US Pennsylvanian women using data from two population-based case-control studies conducted in the 1990s.

MATERIALS AND METHODS

Study subjects

Subjects for the analyses presented here came from two population-based case-control studies undertaken in the 1990's, one in Israel (1, 5) and the other in the United States (7, 8) (see figure 1). For both studies, Institutional Review Board approval was obtained and subjects participated after giving informed consent. The Israeli study identified all Jewish women throughout the State of Israel with pathologically-confirmed cancer of the ovary or primary peritoneal carcinoma diagnosed between March 1, 1994, and June 30, 1999. To ensure that no patients with newly diagnosed cancer were overlooked, all the departments of gynecology in the country were monitored continually throughout the study and pathology and oncology departments were checked monthly. Of the 1707 cases identified, 1443 (84.5%) gave consent and were interviewed. Of those not recruited, 147 (8.6%) died or were too sick to participate, and 117 (6.9%) refused to participate.

For each case, two healthy controls individually matched for age (±2 years), ethnic origin, and place and length of residence in Israel, were recruited using the Israel National Population Registry. The use of this registry ensured that women invited to participate as controls, were representative of the general population. About 67% of all

women asked to participate as controls agreed, achieving a total of 2384 controls. To ensure comparability between studies, and because the age range within the U.S. study was 20-69, this age range restriction was applied to the Israeli data. Thus, this analysis is based on 998 epithelial ovarian cancer cases and 1528 controls.

In the American Study, cases were women age 20-69 diagnosed with incident epithelial ovarian cancer within the six months prior to interview. Between May 1994 and July 1998, 873 eligible women were identified at 39 hospitals around the Delaware Valley. Fourteen physicians did not consent to their patients' participation and 92 women refused to participate. Thus, our analyses are based on the 767 completed case interviews (88% of potentially eligible incident cases). The diagnosis of epithelial ovarian cancer was confirmed by pathology in all cases.

Controls aged 65 or younger were ascertained by random digit dialing and frequency matched to cases by 5-year age groups and three digit telephone exchanges. Of the 14,551 telephone numbers screened for this purpose, we identified 1,637 households with a potentially eligible control, of whom 1215 (74%) completed interviews. Controls aged 65-69 were ascertained through Health Care Financing Administration (HCFA) lists. Of the 263 potentially eligible participants identified, 152 (58%) were interviewed. Therefore, of the 1,900 screened and potentially eligible controls, 1,367 (72%) are included in these analyses.

Data collection

In Israel, face to face interviews were conducted by a group of experienced, multilingual, trained interviewers, and when needed, the interview was conducted in the

native language of the respondent. Cases were interviewed in the hospital, typically four to six days after gynecologic surgery. Controls were interviewed at home by the same highly trained staff that interviewed the cases.

In the American study, a standardized 1.5-hour in-person interview of cases and controls was conducted at the subject's home. In both studies, detailed information was obtained on demographic, anthropometric, lifestyle, hormonal, medical, as well as personal and family history of cancer. Extensive gynecologic and obstetric histories were also obtained. A life calendar, marked by important happenings that participants recalled during their lives, was used to enhance memory of distant events.

Statistical methods

Within each population, frequencies were calculated for demographic variables as well as for potential ovarian cancer risk and protective factors such as: family history of ovarian and breast cancer, reproductive variables, contraceptive and hormonal use, gynecological variables and body mass index (BMI) (weight in kilograms/height in meters^2). Chi-squared tests were used to test for significant differences in the frequencies of categorical risk/protective factors and t-tests for statistical differences in continuous risk/protective factors between Israeli and American controls.

Due to the different matching design of the two studies, the individual matching of the Israeli study was broken and an unmatched analysis was performed. Odds ratios with corresponding 95% confidence intervals were calculated as the primary measure of effect size using univariate unconditional logistic regression with adjustment for age, education and ethnic origin/race. Variables that were significant in the univariate analysis

or that were considered important biologically were entered into multivariate logistic regression analyses. Odds ratios between the two study populations were compared using Wald's Test. Population attributable risks were calculated for each variable using the methods described by Benichou for evaluating attributable risk for categorical and continuous variables (9). Confidence intervals for the attributable risks were calculated using the methods given by Greenland (10).

RESULTS

Table 1 shows the distribution of variables with known risk/protection for ovarian cancer among Israeli and U.S. controls. Significant differences (all at a level of $p<0.001$) were detected for most of these factors between the two groups. With respect to reproductive variables, Israeli women reported a later age at menarche, greater number of live births and proportion of women who ever gave birth, higher percentage of breastfeeding among parous women, longer duration of breastfeeding among ever breast feeders, and later age at menopause. With respect to contraception and use of hormones, Israeli women were significantly more likely to use an IUD (37% vs. 16.7%); and less likely to have a tubal ligation (3% vs. 33%), to use oral contraceptives (28.3% vs. 68.7%), and to take hormone replacement therapy (12.1% vs. 33%). Hysterectomy and use of talc were more prevalent in the US. Surprisingly, Israeli women were significantly less likely than U.S. women to report a family history of both breast (6.2% vs. 10.0%) and ovarian neoplasms (0.8% vs. 2.0%). Body mass index at age 18 was higher by an average of 0.6 units in Israeli women ($p<0.001$).

In both Israel and the U.S., the main histologic subgroup for the cases was serous (40.3% in Israel, 36.5% in the U.S.) (data not shown). This was followed by endometrioid tumors (13.0% versus 17.7%) and mucinous tumors (4.0% versus 6.8%). Other non-specified and mixed tumors accounted for 15% of Israeli and 12.4% of U.S. tumor histologies. Borderline tumors constituted 20.7% and 19.7% of Israeli and U.S. tumors, respectively.

Basic demographic features differed between the study populations (data not shown). Israeli controls were significantly older than American controls (52.3 versus 49.5 years; p=0.0002). About 50% of Israeli participants were of American-European origin as compared to the more than 80% of Americans who were white (p<0.001). The mean number of years of completed education in the Israeli group was 12.7 years, significantly less than the 13.2 mean number of years of education among Americans (p<0.001). To account for these demographic differences, subsequent analyses were adjusted for age, race/ethnic origin, and education.

Table 2 compares odds ratios between the two populations adjusted for age, race/ethnic origin, and education. Both studies confirm the role of well established factors in ovarian cancer risk. However, high parity and oral contraceptive use were more protective among American than Israeli women. Parity was inversely and significantly associated with ovarian cancer in both groups; however, higher parity ($\geq$3 children) was significantly more protective in the American population (OR=0.26 vs. 0.49; p=0.03). Use of oral contraceptives for less than one year, as compared to never use of oral contraceptives, was associated with a borderline significant increase in risk in the Israeli but not in the U.S. population. This difference between the two nationalities was

significant (p=0.01). About 78% of the women who used oral contraception for up to 1 year were, in fact, short-term users (≤6 months). When this latter category was included in the reference group, the increased risk seen in the Israeli study for the lowest category of use disappeared (data not shown). Ten or more years of oral contraceptive use was significantly protective in the US population (OR=0.36; 95% CI 0.23-0.57), but not among the Israelis (OR=0.71; 95% CI 0.39-1.29), a difference of borderline significance (p=0.07) that might reflect the small number of long-term users in Israel (25 women among the cases and 45 among the controls). While a high BMI (≥30) at age 18 was not associated with ovarian cancer in the US group, a high BMI at age 18 was associated with an odds ratio of 2.94 among Israeli women.

No other odds ratios differed significantly between the two populations. In both populations, family history of ovarian cancer was the strongest risk factor (OR=6.17 and 3.17 for Israel and the US, respectively) and did not differ between populations (p=0.15). Family history of breast cancer was a significant risk factor only in the Israeli population (OR=1.54) but was not significantly different between populations. In both groups, a non-significant protective effect was observed for hysterectomy and IUD use. Tubal ligation was protective and talc use a risk factor in both groups but only significantly so in the American group. Hormone replacement therapy use was not a risk factor in the US population, and was associated with a 28% increased risk of borderline significance in the Israeli population. Age at menarche was not associated with ovarian cancer in either population.

The top three factors contributing to the PAR among Israelis and Americans were relatively similar (Figure 2). For Israelis, it was lack of hysterectomy, lack of tubal ligation,

and fewer than 10 years of oral contraceptive use. For Americans, it was fewer than 10 years of oral contraceptive use, lack of tubal ligation, and fewer than three live births. Significant differences in the magnitude of the population attributable risks between the groups were noticed for live births and talc use which were both more pronounced in the American group. Although non significantly different from the Israeli group, the PAR of not breastfeeding, not going through tubal ligation, not using IUD and using OC for less than 10 years, were greater in the American group.

DISCUSSION

In the case- control studies from Israel and the U.S. presented here, Americans, on the whole, had a greater prevalence of contraceptive protective factors including oral contraceptives and tubal ligation. Lack of these same protective factors were among the three top contributors to the PAR in both populations, but more so for Americans. In other words, American women tended to be more protected by these known exposures than Israeli women. This is surprising and opposite to our *a priori* hypotheses that Israelis would have a greater frequency or a greater protection from exogenous factors, thereby countering their excess genetic risk and accounting for the similarity in overall ovarian cancer incidence between U.S. and Israeli populations.

Reassuringly, our data generally confirm the risk and protective factors previously reported to be associated with ovarian cancer. Oral contraceptives are considered to be the most powerful known chemopreventative agents for ovarian cancer with longer use affording greater protection (11-13). Parity has been shown to be protective for ovarian cancer in a plethora of studies with first birth affording the greatest protection and each

additional birth adding to the protection (13-17). Finally, tubal ligation consistently reduces the risk of ovarian cancer in many studies (18).

Two large case control studies have investigated the possible interaction between *BRCA1/2* mutations and some of the aforementioned factors (1, 19). Both studies demonstrated the protective effect of parity among both carriers and non-carriers. However, while Narod et al showed a protective effect of oral contraceptives among carriers, Modan et al, from Israel, did not find that oral contraceptive use was protective in carriers. In the current study, we found protection from parity, oral contraceptives and tubal ligation in both populations, but the protective effect of high parity and of long-term oral contraceptives were both weaker among Israelis. This is at least partially consistent with the Modan et al. finding that in Israel, with its higher prevalence of *BRCA1/2* carriers, the protection afforded by oral contraceptives is moderated. However, our observation counters the expectation that the known exogenous factors might provide greater protection among Israelis.

While our findings are provocative, certain limitations must be considered. The major assumed difference between the two populations is the prevalence of *BRCA1/2* mutations. While the Israeli study directly tested carrier status of the *BRCA1/2* founder mutations among women with ovarian cancer and found that about 30% of women to carry a mutation, the prevalence of *BRCA1/2* carrier status in the American population remains unknown. However, studies in similar populations estimate the prevalence to be about 10% (1, 20, 21). Similarly, the exact rate of Jewish origin among the American group is unknown, although a small subset of participants for whom religion was obtained suggests that the rate is less than 10%.

Family history of ovarian and/or breast cancers does not correlate well with *BRCA1/2* carriage (20). Indeed, an unexpected finding in our comparison was the smaller proportion of Israeli rather than American women reporting a family history of ovarian and breast neoplasms. Misclassification of family history may be attributed to various factors, including early truncation of Jewish families due to the Holocaust, which causes an underreporting of familial cases because first degree relatives may not have survived long enough to develop ovarian cancer. Underreporting of familial cases could also result from paternal inheritance of the mutation or incomplete penetrance. The existence of other unidentified ovarian cancer genes may account for the cases of family history observed in non-*BRCA* carriers. Furthermore, limited data on age of onset for family members with breast cancer in this study could cause an overestimation of familial aggregation because breast cancer is so common (6). That is, family cases among older relatives are likely to occur by chance and may not necessarily suggest a familial cluster. Therefore, it is not entirely appropriate to consider family history as a proxy for *BRCA1/2* carriage. Nonetheless, we observed a substantial increase in risk associated with a family history of ovarian cancer in both populations.

Another limitation of our study is lack of information about other putative risk/protective factors for ovarian cancer. For instance, we do not have comparable data on non-steroidal anti-inflammatory medications or diet. However, to date, studies have not consistently shown an association between these factors and ovarian cancer (22-25).

A final limitation is the lack of standardization in questionnaire design and implementation between the two studies. Although the questions asked were very similar, they were not identical and although questionnaires were delivered by trained

interviewers, the training was not common to the two studies. Therefore, methodologic differences between the studies may confound our results.

Given that we could not explain the similar rates of ovarian cancer between Israelis and Americans despite the greater genetic predisposition on the part of the former group, we must consider other explanations. The most apparent of these is the existence of undiscovered gene-gene and gene-environment interactions. It is likely that other genes affect ovarian cancer risk; each of these probably has a lower penetrance than *BRCA*, and each probably accounts for a smaller portion of ovarian cancer risk. Nonetheless, these might potentially account for some of the risk among American non-Jews. Exposures not measured in this study and not clearly related to ovarian cancer may also explain the surprisingly low rate of ovarian cancer among Israelis. In particular, whereas 36% of Ashkenazi Jewish women with epithelial ovarian cancer in Israel will carry a mutated *BRCA1/2* gene (5), over 45% of Jewish American women with ovarian cancer will be carriers (14). The greater penetrance of *BRCA1/2* in the US supports the existence of an unidentified environmental or lifestyle factor. For example, because of its lower latitude, sun exposure is greater in Israel than in Pennsylvania, suggesting that Vitamin D exposure may be protective against ovarian cancer, just as it may be for other hormonally-linked cancers including breast (26) and prostate (27) cancers. Notably, in the US ovarian cancer mortality declines as one moves from north to south (28) and international data for westernized countries suggests a similar latitude-related trend in ovarian cancer incidence (6). Moreover, ecological studies in the US have shown an inverse association between sunlight exposure and ovarian cancer mortality, (29, 30), although no well-designed studies have specifically assessed the sunlight-ovarian cancer

association. Another possible exposure difference between Americans and Israelis is the over-sanitation in the American environment. It has been proposed that lack of exposure to a variety of bacterial and viral antigens in childhood may weaken tolerance within the immune system and cause a variety of autoimmune diseases. Given the inflammation hypothesis of ovarian cancer (31), one explanation is that this difference in environmental sanitation might provide an excess of ovarian cancer cases among Americans.

Ovarian cancer is a virulent and often fatal disease because it is usually detected at a very late stage. Identifying risk factors represents the most promising way to support prevention and early detection efforts, thereby increasing survival. Our data support the existence of unknown genetic, environmental and/or lifestyle factors with important impact on risk and compels us to identify these missing links in the etiology of ovarian cancer.

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TABLE 1. Comparison of potential risk/protective factors between the controls of the Israeli & the U.S. groups

	Israeli N=1528		American N=1367	
I. 1st degree family history (n,%)				
Positive breast cancer	95	6.2	140	10.0*
Positive ovarian cancer	12	0.8	25	2.0†
II. Reproductive variables				
Age at menarche (mean±SD)	13.2±1.5		12.6±1.6*	
Live births (mean±SD)	2.8±1.8		2.4±1.8*	
Breastfeeding (yes)				
For the total group (n,%)	1171	76.6	613	44.8*
For women with ⩾1 live birth (n,%)	1171	81.9	613	51.5*
Duration, for women who ever breastfed (month) (mean±SD)	18±28.6		12.4±14.4*	
Age at natural menopause (mean±SD)	49.7±4.5		46.2±7.2*	
III. Contraceptive use & hormonal variables (n, %)				
IUD (yes)	562	37.0	228	16.7*
Tubal ligation (yes)	46	3.0	451	33.0*
Oral contraceptive use (yes)	430	28.3	939	68.7*
Hormone replacement therapy use (yes)	185	12.1	445	33.0*
IV. Gynecological variables (n, %)				
Hysterectomy (yes)	120	7.9	181	13.0*
Talc use (yes)	74	4.8	219	16.0*
V. Body Mass Index (kg/m^2) (BMI) at age 18 (n,%)				
<20	441	36.8	606	44.7*
20-<25	644	53.7	647	47.8
25-<30	106	8.8	75	5.5
≥30	9	1.0	27	2.0

** $p<0.001$, † $p=0.02$*

TABLE 2. Unconditional Multivariate Odds Ratios (OR) for OvC by population adjusted for age, education & race/ethnic origin

		Israeli		American		
		OR	**95% CI**	**OR**	**95% CI**	**P Value***
I. Positive 1st degree family history						
Breast cancer (vs. no)		1.54	1.08-2.22	1.13	0.83-1.52	0.2
Ovarian cancer (vs. no)		6.17	3.0-12.69	3.17	1.80-5.57	0.15
II. Reproductive variables						
Age at menarche (continuous)		0.96	0.90-1.03	1.03	0.97-1.10	0.12
Live births (vs. 0)	**1**	0.62	0.39-1.01	0.47	0.34-0.67	0.36
	2	0.52	0.33-0.82	0.33	0.24-0.45	0.11
	3+	0.49	0.31-0.78	0.26	0.19-0.37	0.03
Breastfeeding (vs. no)		0.97	0.75-1.26	0.84	0.67-1.05	0.41
III. Contraceptive use & hormonal variables (years)						
IUD (vs. no)		0.93	0.74-1.16	0.84	0.63-1.12	0.58
Tubal ligation (vs. no)		0.73	0.40-1.34	0.60	0.47-0.76	0.55
Oral contraceptive (vs. no)	**<1**	1.6	1.06-2.41	0.81	0.61-1.07	0.01
	1-9	0.82	0.63-1.07	0.79	0.62-1.01	0.84
	10+	0.71	0.39-1.29	0.36	0.23-0.57	0.07
Hormone replacement therapy (vs. never)		1.28	0.96-1.71	0.99	0.80-1.23	0.17
IV. Gynecological variables						
Hysterectomy (vs. no)		0.65	0.44-0.98	0.82	0.60-1.12	0.37
Talc (vs. no)		1.11	0.72-1.7	1.38	1.08-1.76	0.39
V. Body Mass Index (BMI) at age 18 (vs. <20)						
	20-24	1.14	0.92-1.41	1.18	0.97-1.45	0.82
	25-29	1.62	1.16-2.28	1.47	0.99-2.19	0.71
	≥30	2.94	1.26-6.89	0.62	0.29-1.29	0.01

* Based on Walds test between America and Israel

Figure 1:

Description of the study population by study group (Israel and USA)

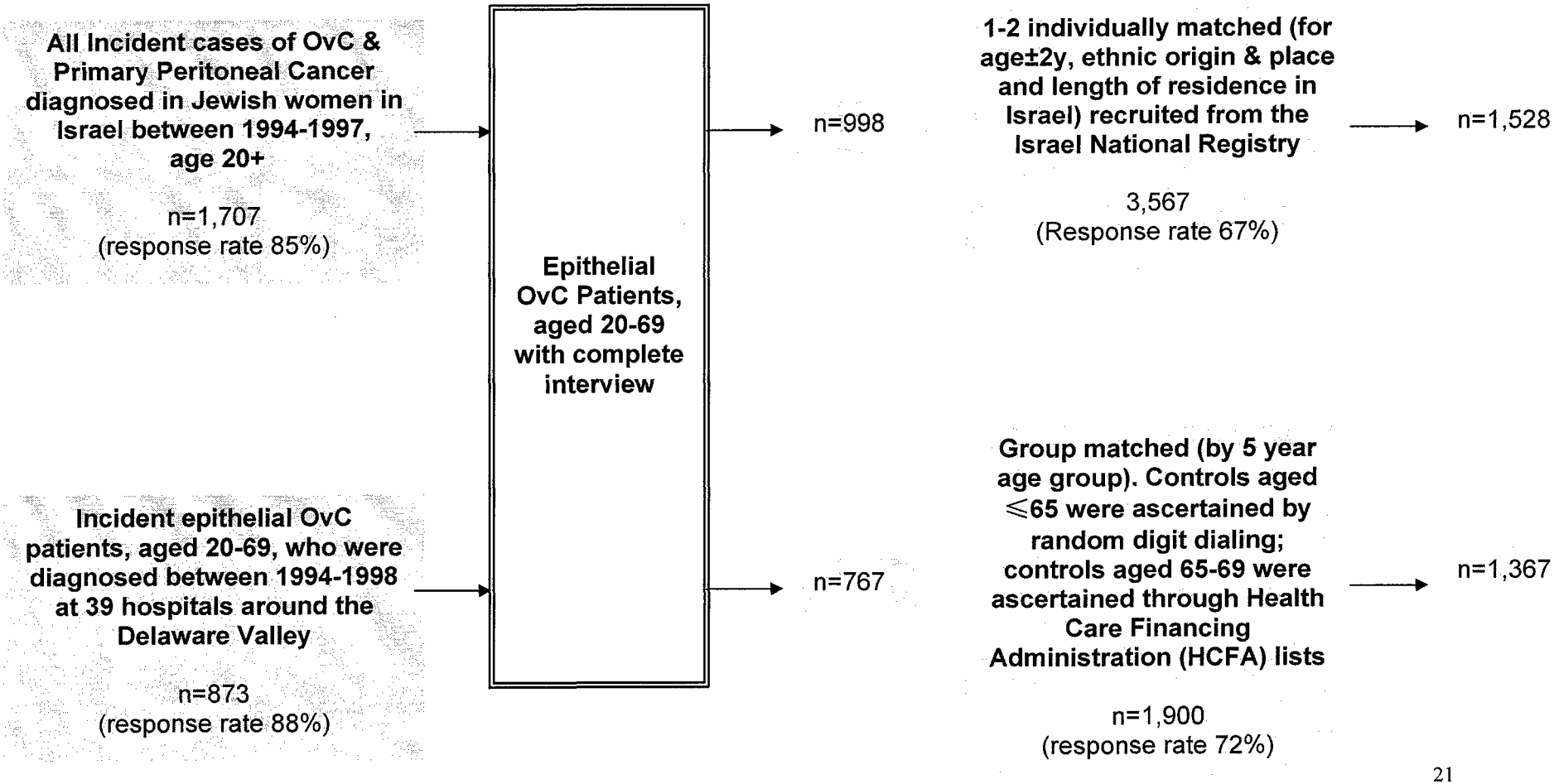

Figure 2:

Population Attributable risk (PAR) based on multivariate adjusted Odds Ratios for OvC